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# Pathogenic Biofilms as Triggers of Recurrent Vaginitis and Cystitis

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## SUMMARY

This paper focuses on biofilms and their role in chronic infectious diseases and human health. Pathogenic biofilms can be intracellular and extracellular. They interact with host's cells at different levels of aggressiveness and complexity. Pathogenic biofilms are the neglected etiology of recurrences in various medical fields, including cardiology, pneumology otorhinolaryngology, gastroenterology and urogynecology. The indiscriminate use of antibiotics has caused a dramatic escalation of microbial aggression and antibiotic resistance, linked to pathogenic biofilms also in urology and gynecology. As a consequence, new diagnostic and therapeutic challenges are emerging in the clinical practice.

D-mannose, a bioidentical sugar, deriving from both exogenous and endogenous sources, can be considered an useful therapeutic molecule. Increasing evidence supports its role in the prevention and treatment of pathogenic biofilms in recurrent vaginitis and cystitis. It should be considered in the multimodal prevention and treatment of urogynecological antibiotic-resistant recurrences.

**Keywords**: pathogenic biofilms, D-mannose, persister cells, recurrent Urinary Tract Infection (rUTI), antibiotic resistance, vaginitis.

 

# INTRODUCTION

Pathogenic biofilms are the emerging frontier of research and clinical strategies to face the challenging issue of increasing antibiotic resistances. In urology and gynecology, recurrent cystitis and vaginitis represent an important part of daily medical consultations. The failure of a clear diagnosis is often frustrating both for patients and physicians. The aim of this short study is to review the evidence on pathogenic biofilm and to consider new non antibiotic strategies of intervention.

## *Structure of biofilms and their scenario in humans*

Biofilms are composed by germs aggregated within a self-produced matrix. About 15% of biofilms composition is a complex assembly of synergistic and often pathogenic microorganisms, secreting approximately 85% of adhesive polysaccharides (Extracellular Polymeric Substance, EPS). The biofilm has a primitive circulatory system. The structured communities of bacterial and fungal cells are enclosed in a self-produced polymeric matrix and adhere to a living or inert surfaces, such as medical devices [1,2]. Inside the biofilm, microorganisms offer a passive resistance to environmental damaging factors, immune system and antibacterial substances both present in the environment and in host organism during infections [2]. The amplified bacterial resistance compared to planktonic forms fall into three basic mechanisms:

**1**. physical-chemical resistance (ciprofloxacin and aminoglycosides, that usually reach the target in few seconds, take up to 21 minutes to reach bacteria inside the biofilms);
**2**. metabolic resistance (slow metabolic cell profile);
**3**. genetic resistance (plasmids transfers) [3].

Biofilm are involved in diseases in diverse medical specialties, including but not limited to: endocarditis, recurrent bronchial or lung infections, rhinosinusitis, otitis media, prostatitis, gastrointestinal bowel disease, cystitis and vaginitis underlying a general microbiologic aggressive and survival-oriented strategy. Table 1 summarizes the microbial etiology of biofilms involved in different human diseases [4-8].

In uroginecology the recent increase of bacterial resistance and microbial aggression suggests a connection with the indiscriminate use of antibiotics and pathogenic biofilms' formation in vagina and in urothelium [2,9,10]. Therefore, an innovative approach is necessary to avoid the escalation in antibiotic use and antibiotic resistances in urogynecology [11].

**Table 1.** Biofilm in various human organs and systems.

| Infection | Predominant bacteria in biofilm |
|---|---|
| Otorhinolaryngology | *Staphylococcus aureus*, *Streptococcus pneumonia, Haemophilus influenza, Moraxella catarrhalis* |
| Gastrointestinal tract | *Enteric bacteria* (*E. coli*) |
| Endocardium | *Streptococci, Staphylococci, Fungi* (*Aspergillus, Candida* spp.) |
| Respiratory tract | *Pseudomonas aeruginosa* (CF suffering patients) |
| Stomach | *Helicobacter pillory* |
| Vagina | *E. coli* |
| Prostate | *E. coli and E. faecalis* |
| Urinary tract | *Uro Pathogenic E. coli* (UPEC), *Ureaplasma urealiticum*, *Proteus*, *Pseudomonas* spp. |

# METHODS

This paper briefly reviews the pathophysiology of biofilms as novel contributors to recurrent human infections. The literature research was focused on the following key words: biofilms, pathogenic biofilms, recurrent cystitis, recurrent vaginitis, antibiotic resistance, extra- and intracellular biofilm, biofilm in chronic diseases. It focuses on understanding their crucial role in recurrent urogynecological infections and considering new preventive and therapeutic strategies.

# RESULTS

In the urogynecological field, recidivism and chronic infections can be explained through the theory of biofilms. In the vagina and in the urothelium biofilms can be respectively extracellular and intracellular.

In both cases, in the deep layer of biofilms, with a low presence of oxygen and nutrients, pathogens live in a quiescent state as ***persister cells*** **(PC)**. They are resistant to antibiotics because they are in a phenotypic and metabolic protected state. The deep location of PC also ensures bacterial cells resistance to innate and cell mediated immune host defenses, but always ready to re-attack the host. The persistence of vaginitis and cystitis, with alternate phases of

bacteriuria, are justified through the detachment of a persistent bacterial inoculum from a mature biofilm that colonizes other venues, until systemic invasion. Diagnosis and treatment of biofilms may be more efficient if compared to the antibiotic therapy, often ineffective in the long term [2,12,13].

The knowledge of biofilms can help physicians to explain:

1. incomplete or absent response to prolonged antibiotic therapy;
2. high presence of co-morbid forms of antibiotic-resistant infections and diseases;
3. the increasing bacterial resistance to immune effectors;
4. the infection's tendency to become chronic [14].

**Extracellular vaginal biofilms** usually grow close to the vaginal vestibule, and long the vaginal wall. They reside near apical cell surface of the vaginal mucosa and protrude towards the cavity [1,2,15]. Extracellular biofilms are characteristic of the surface of different mucous or waste materials, such as medical devices (vaginal contraceptive ring, pessary, intrauterine devices, subcutaneous implants, catheters). In urogynecology they give rise to antibiotic-resistant chronic polymicrobial infections and explain recidivism and comorbidities between recurrent cystitis and vulvar vestibulitis with provoked vestibulodynia [2,9].

**Intracellular biofilm** resides inside the urothelium that covers the inner bladder/wall bladder (Fig. 1). They are characterized by a specific pathogenic strain of *Escherichia coli*, carrier of the antigen K (UPEC). This strain is responsible for 75-85% of recurrent cystitis and intracellular biofilm formation [16,17]. Intracellular bacteria are enclosed in a polysaccharide matrix and enveloped by a protective shell of uroplakins [18]. Intracellular biofilms are a reservoir of microorganisms immune to antibiotics and to immune system effectors. Mimicking what happens in the outer world, they are equivalent of intracellular "terrorists" difficult to be reached by antibiotics and the host' immune system, and yet capable of progressive tissue damage and inflammation. They cause chronic inflammation of the bladder wall, which can evolve to cause a "painful bladder syndrome", and "interstitial cystitis" [19,20].

## *Comorbidity in urogynecology*

Recent evidence stresses the synergism and the increasing comorbidity between recurrent cystitis, recurrent vaginitis, introital coital pain and its leading etiological counterpart, that is **provoked vestibulodynia** (**PVD**). It was formerly defined as vulvar vestibulitis [9]. Comorbidity in urogynecology also supports the need of a parallel vision to address recurrent vaginitis and cystitis.

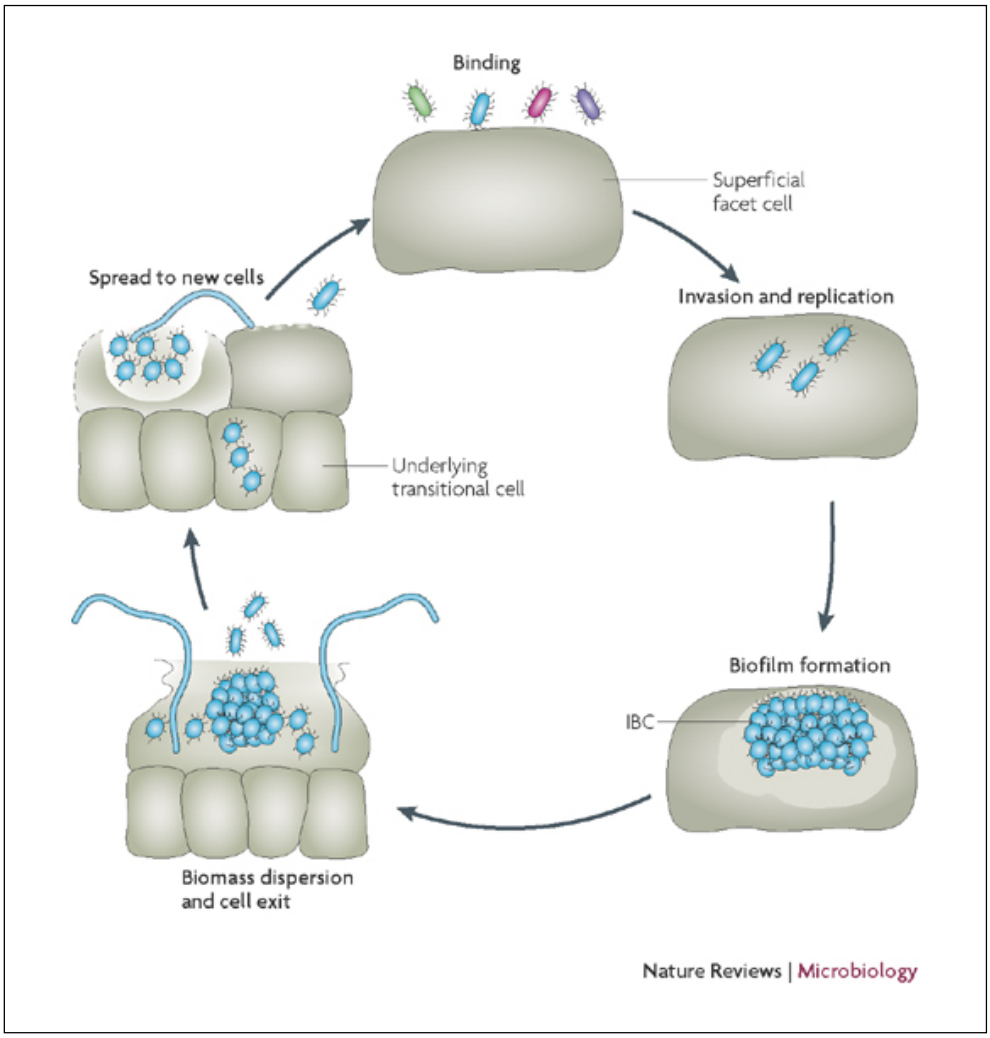


**Figure 1.** Schematic representation of bladder intracellular biofilm. Uro-Pathogenic *Escherichia coli* can invade and replicate inside bladder cells constituting Intracellular Bacterial Communities (IBCs). From L. Cegelski et al., 2008[17].

Salonia et al., in their study on 60 consecutive patients with mean age 34.2 years (median 33 years; range 21-42) complaining of recurrent cystitis, documented that:

- 36 of 60 patients (60%) were suffering from secondary PVD;
- women with PVD had a higher prevalence of urinary tract infections

(UTIs) over the previous 12 months ($\chi^2$: 4.54; P = 0.03) and suffered more frequently from UPEC-related rUTIs ($\chi^2$: 5.92; P = 0.01) than those without PVD;

- women with PVD showed significantly lower scores on Female Sexual Function Index domains (all P < 0.01), as compared with PVD-negative women. UPEC-related rUTIs (odds ratio [OR]: 3.1; P = 0.01);
- six or more UTIs were reported over the previous 12 months (OR: 2.8; P = 0.01), and treatment with three or more antibiotics throughout the same period (OR: 2.1; P = 0.04) emerged as independent predictors of PVD.

*E. coli* has been documented in vagina up to 14 days before a cystitis. Laumann et al. in 1999 documented that women with lower urinary tract symptoms have an OR of 7.61 to perceive coital pain ("introital dyspareunia"). The coital trauma may trigger the activation of *E. coli* intracellular bacterial communities through a mechanical and inflammatory mechanism [21].

The importance in investigating alternative therapeutic agents to antibiotics is triggered by increasing values in bacterial resistances. Recent data obtained in an epidemiological study carried out between January 2008 and September 2010, showed that *E. coli* UPEC isolated from recurrent UTI suffering patients was resistant to multiple antibiotics: 77.56% multidrug resistant to at least three antibiotics, 55.60% to at least five antibiotics. The highest resistance rate, 87.32% was shown by Piperacillin, followed by gentamicin 75.12%, levofloxacin 70.73%, and cefazolin 61.95%. However, the sensitivity to amikacin, piperacillin/tazobactam and ceftazidime was comparable (respectively 9.31%-5.88%-9.76%). Also β-lactamases production in *E. coli* was investigated and the results showed that 20% of *E. coli* strains had a significantly higher resistance and recurrence rates [22].

**A different preventive prophylactic and therapeutic strategy** is possible and it is currently focused on preventing mucosal bacterial adhesion and biofilm formation. These **alternatives to antibiotics** can be found in **bio identical** substances, even tissue reparative, such as D-mannose (Box 1) [2]. This strategy, integrated in a multimodal approach, may help women to prevent recurrent vaginitis and cystitis.

**Box 1. D-mannose: the biological basis of its efficacy**

**Exogenous D-mannose** is extracted from birch and larch wood. It is a **bioidentical substance, as it has the same structure of endogenous D-mannose**, a low molecular weight monosaccharide, that is a normal component of different biological molecules. When ingested, D-mannose is sparsely meta-

bolized by human cells and it is eliminated unchanged through the urine. D-mannose has an high affinity to lectins of *Escherichia coli* and to many other fimbriated bacteria. The use of D-mannose seems to be a winning strategy in both prophylaxis and interventions in recurrent urinary tract infections (rUTI), as it prevents bacterial adhesion and facilitates their mechanical detachment (Fig. 2) [23,24].

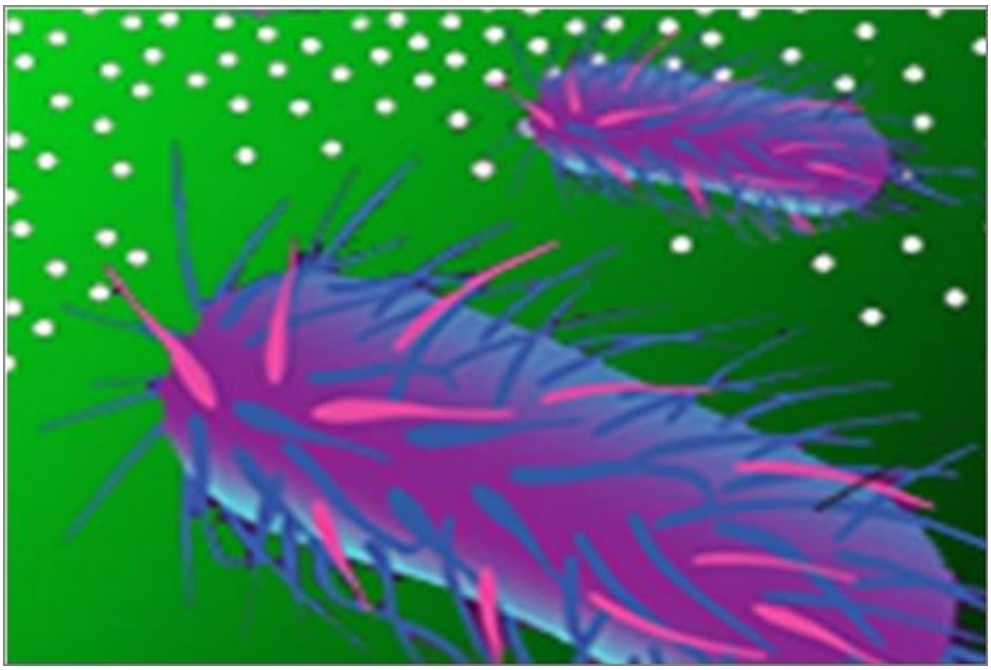

**Figure 2.** D-mannose action against *Escherichia coli***.** D-Mannose, shown here with white balls, binds the tip of *E. coli* fimbriae and prevents urothelial and bladder surface cells adhesion. Image kindly obtained by Dr. D'Errico G.

Exogenous **D-mannose** mimicks, surrogates and **integrates a protective kidney protein, the Tamm-Horsfall protein**, usually defective in women more vulnerable to recurrent cystitis [25].

D-mannose prevents *E. coli* cell receptors to adhere on bladder and vaginal epithelium, first stage of cystitis, vaginitis and biofilm formation. D-mannose also favors the restructuring of damaged mucosa, especially of the vagina, thus ensuring greater protection from subsequent bacterial and mechanical damage [26,27].

# CONCLUSION

Today neither antibiotics nor antifungal substances are able to prevent biofilm formation. A better understanding of dynamics and complex organization of those bacterial consortia, both physiological and pathological, is essential in order to limit the use of antibiotics. To reach this goal, it is important to have a global vision of microbial alliances and synergies, in order to "win without fighting". In this complex scenario, increasing evidence supports the role of D-mannose in the prevention and treatment of recurrent vaginitis and cystitis. Exogenous D-mannose, behaving like a bio identical substance, mimicks and integrates a protective kidney protein, the Tamm-Horsfall protein, defective in women more vulnerable to recurrent cystitis. D-mannose can open up new promising possibilities for an integrated approach, in order to avoid, whenever possible, more conventional antibiotic prophylaxis and treatments.

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Conflicts of interest:
**Prof. Alessandra Graziottin, MD**:
Speakers' bureau: Abbott, Bayer, Deakos, Lolipharm, Menarini, Pfizer.
Advisory Boards: Bayer, Menarini.
Consultant: Abbott, Bayer, Deakos, Epitech, Menarini, Pfizer.
**Dott. Pier Paolo Zanello**: Consultant: Deakos.